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Design of a new DNA-polyintercalating drug, a bisacridinyl peptidic analogue of Triostin A

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The synthesis of a new bifunctional compound in which two aminoacridine chromophores are linked by the bicyclic depsipeptidic backbone of des-*N*-tetramethylTriostin A is described. The molecule, bis-[(9-acridinyl)-D-seryl-L-alanyl-L-cysteinyl-L-valine] dilactone disulphide, structurally analogous to the antibiotic anti-tumour drug Triostin A, is shown to possess a high affinity to DNA and to act as a bis-intercalator on the basis of spectroscopic, viscosimetric and thermal-denaturation studies. This model constitutes the first attempt of a synergic association between a peptidic moiety that mimics a naturally occurring drug and aminoacridine, the two parts themselves each exhibiting a high affinity for the DNA target.

The concept of bis-intercalation of anti-tumour drugs in which two planar chromophores are connected by various linker chains has gained considerable interest (Waring, 1981, and references cited therein). Among the numerous candidates as monomeric heterocyclic molecules for the building of synthetic bis-intercalating compounds, 9-aminoacridine has often been chosen because of its biological properties, attributed to its binding to DNA (Albert, 1966; Blake & Peacocke, 1968; Muller *et al.*, 1973). The most extensive studies on diacridines have dealt with compounds having two acridine chromophores linked by a diaminoalkyl chain of various lengths (Le Pecq *et al.*, 1975; Barbet *et al.*, 1976; Canellakis *et al.*, 1976*a*,*b*,*c*; Canellakis & Bellantone, 1976; Fico & Canellakis, 1977; Wakelin *et al.*, 1976). If the alkylated chain is long enough, it can be envisaged that there is a folding of the molecule, facilitating the intercalation of the acridine chromophores between DNA base-pairs, whereas the drug should be extended when studied in solution. Several authors have studied this question (Le Pecq *et al.*, 1975; Wakelin *et al.*, 1978), and they have come to the conclusion that a minimal interchromophore distance of 0.88nm (8.8Å) is favourable to bis-intercalation. However, the intrinsic association constants measured (Wakelin *et al.*, 1979) for these compounds are rather weak. Therefore we decided to build a new bifunctional compound in which the two aminoacridine moieties are linked by the bicyclic depsipeptidic backbone of the des-*N*-tetramethylTriostin A (TANDEM). Des-*N*-tetramethylTriostin A, an analogue of the well-known anti-tumour antibiotic Triostin A (Otsuka & Shoji, 1967; Otsuka *et al.*, 1976; Chakravarty & Olsen, 1978) composed of two 2-quinoxalinecarbonyl moieties attached to a symmetrical octapeptide bis-(D-seryl-L-alanyl-L-cysteinyl-L-valine), has been shown to possess a high binding constant for DNA and a strong preference for (A-T)-rich DNA sequences (Lee & Waring, 1978). The peptide portion binds to DNA via its minor groove, and the two well-positioned quinoxaline rings form a two-base-pair sandwich with an interchromophore distance of 1.19nm (11.9Å), as determined by X-ray crystallography (Hossain *et al.*, 1982). Nevertheless it is interesting to note that the single heterocyclic quinoxaline-2-carboxamide is not able to intercalate between the DNA base-pairs (Wakelin & Waring, 1976). Only the peptidic backbone plays a major role in the strength of the DNA binding, and this was the reason why we replaced the quinoxaline rings by aminoacridine chromophores. In this model compound (Fig. 1) the rigid peptide portion induces steric constraints that should place the two aminoacridine rings in ideal positions to form a two-base-pair sandwich favourable to an increase of the binding constant and a possible sequence specificity.

Abbreviations used: BSACV, bis-(D-seryl-L-alanyl-L-cysteinyl-L-valine) dilactone disulphide; BASACV, bis-[(9-acridinyl)-D-seryl-L-alanyl-L-cysteinyl-L-valine] dilactone disulphide.

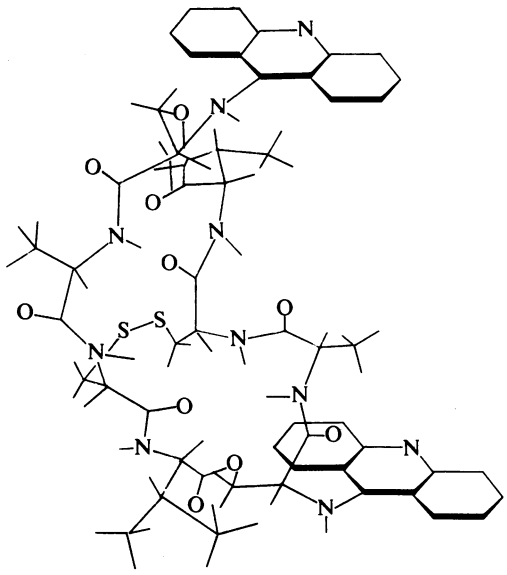

Fig. 1. *BASACV*

Experimental

The bicyclic octapeptide bis-(D-seryl-L-alanyl-L-cysteinyl-L-valine) dilactone disulphide (BSACV) was synthesized by the liquid-phase method according to a procedure previously reported (Ciardelli *et al.*, 1978). The peptide was judged to be pure on the basis of its amino acid analysis after hydrolysis with 5.6M-HCl and t.l.c. in chloroform/ethanol (4:1, v/v). The two acridine rings were introduced on the $N_{(\alpha)}$ atom of the serine residues by refluxing an equimolecular mixture of BSACV dihydrobromide and of 9-chloroacridine in methanol for 12h. After neutralization with dilute ammonia, the expected bis-[(9-acridinyl)-D-seryl-L-alanyl-L-cysteinyl-L-valine] dilactone disulphide (BASACV) was purified by column chromatography on silica gel with methanol as eluent. The pure product gave only one spot in t.l.c. [BASACV R_F 0.78 and BSACV R_F 0.03 in chloroform/ethanol (4:1, v/v) solvent system]. Found: C, 59.2; H, 5.2; N, 13.9. Calc. for $C_{54}H_{60}N_{10}O_{10}S_2$: C, 60.4; H, 5.6; N, 13.0%.

Calf thymus DNA (type I, highly polymerized; Sigma Chemical Co.) was used throughout the experiments. U.v. and fluorescence measurements were conducted in phosphate buffer, pH6.9, whereas viscometry experiments were done in 0.01 SHE buffer (9.4mM-NaCl/2mM-Hepes/10μM-EDTA buffer, pH7.0) as described by Wakelin & Waring (1976).

Adsorption coefficients and 'melting' curves were measured by using a Uvikon Kontron 810/820 spectrophotometer coupled to a Uvikon Recorder 21 and a Uvikon Thermoprinter 48. Samples were placed in a thermostically controlled cell-holder (10mm path length). The cuvette was heated by circulating water from a Haake unit set. The temperature inside the cuvette was monitored by using a thermocouple in contact with the solution. The absorbance at 260nm was measured over the range 20–95°C with a heating rate of 1°C/min. The 'melting' temperature T_m was taken to be the mid-point of the hyperchromic transition.

Fluorescence spectra were recorded on a Jobin-Yvon J.Y.3 spectrophotometer.

Viscometry measurements were made by using a Ubbelohde semi-micro dilution viscometer. Temperature was maintained at 20±0.01°C in a thermostatically controlled water bath. Flow times were electronically measured to an accuracy of 0.1s (Schott ABS/G type detector). Calf thymus DNA was sonicated as described by Wakelin & Waring (1976). Solutions were filtered through 0.45μm Millipore filters before measurements.

Results and discussion

Spectroscopic measurements on BASACV solutions were run in the absence and in the presence of calf thymus DNA and compared with those of a corresponding solution of 9-aminoacridine (Table 1). BASACV exhibited a smaller hypochromic effect than did other diacridines. This should be consistent with an intramolecular stacking of the chromophores (King *et al.*, 1982), and has to be related to the rigid structure of the peptidic portion of des-*N*-tetramethylTriostin A. Moreover, it exhibited a hypsochromic effect for the absorption of the acridine rings. This is in accordance with previous reports on bifunctional derivatives of 9-aminoacridine, for which it was demonstrated that the blue-shift is due to a transannular electronic effect between the two aromatic moieties in parallel plane conformation (Himel *et al.*, 1979). On the other hand, intercalation was shown to occur through addition of increasing concentrations of DNA in phosphate buffer, pH6.9, to 9-aminoacridine and BASACV, which showed the typical red-shift and hypochromic effect associated with intercalation.

The interaction of the two 9-aminoacridine derivatives with calf thymus DNA was also studied by fluorescence experiments. In Table 1 are reported the $\lambda_{max.}$ of the emission spectra of the compounds (excitation at 390nm), spectra characterized by a low fluorescence intensity of the bis-acridine derivative. The poor intensity in fluorescence of aminoacridine derivatives substituted in the 9-position has been reported particularly for compounds bearing amide or sulphonamide

Table 1. *Interaction of 9-aminoacridine and BASACV with calf thymus DNA*

	Visible absorption				Fluorescence emission		
	Free		Bound				
Compound	$\lambda_{max.}$ (nm)	$10^{-3} \times \varepsilon_f$ ($M^{-1} \cdot cm^{-1}$)	$\lambda_{max.}$ (nm)	$10^{-3} \times \varepsilon_b$ ($M^{-1} \cdot cm^{-1}$)	Free $\lambda_{max.}$ [nm (relative intensity)]	Bound $\lambda_{max.}$ [nm (relative intensity)]	ΔT_m (°C)
9-Aminoacridine	423	8.1	430	4.30	438 (1)	438 (0.1)*	8
	398	10.1	407	5.25	456 (0.8)		
	382	6.1	387	3.20	480 (0.4)		
BASACV	403	6.2	403.5	5.05	420 (0.45)	420 (0.27)†	14
	385	6.7	389	4.97	440 (0.3)		
					464 (0.1)		

* Quenching obtained with 0.5 mM-DNA for a 10 μM solution of 9-aminoacridine.
† Quenching observed with 5 mM-DNA for a 10 μM solution of BASACV.

groups (Gormley *et al.*, 1978; Gormley & Cysyk, 1979; Hudecz *et al.*, 1981). Concentrated calf thymus DNA solution in phosphate buffer, pH 7, was added in small portions to solutions of 9-aminoacridine and BASACV. After each addition, the intensity of the fluorescence emission was recorded and compared with the signal of the drug in the absence of DNA. The fluorescence of 9-aminoacridine (10 μM) was strongly quenched, indicating a total intercalation in DNA at a concentration of 0.5 mM. The quenching of fluorescence of BASACV was more moderate for the same concentration. A 10-fold concentrated solution of DNA (5 mM) was necessary to observe a similar quenching. This difference could be attributed to a self-association in solution by stacking of the aromatic rings of the bifunctional molecules.

In order to ascertain bis-intercalation, the viscometric behaviour of linear sonicated DNA in the presence of BASACV was studied. The helical lengthening results in an increase in the viscosity of the linear DNA complex according to the approximate relationship (Cohen & Eisenberg, 1969):

$$L_1/L_0 = (\eta_1/\eta_0)^{\ddagger} = 1 + nr$$

where the subscript 1 refers to the DNA complex, the subscript 0 refers to uncomplexed DNA, L is the DNA contour length, η is the reduced specific viscosity, and r is the ratio of bound drug to DNA phosphate; $n = 2$ for a mono-intercalator, and $n = 4$ for a bis-intercalator. The viscometric study of the interaction between sonicated calf thymus DNA and BASACV at pH 7.0 gave a linear plot up to $r = 0.15$, with a slope value comparable with the one exhibited by the diacridine *NN'*-di-(9-acridyl)-1,6-diaminohexane (Wakelin *et al.*, 1978), thus indicative of a bis-intercalative process.

The effect of 9-aminoacridine and BASACV on the thermal denaturation of DNA was assessed by the usual classical technique. Thermal-denaturation profiles of calf thymus DNA were analysed in the absence or in the presence of the two acridine derivatives (Table 1). The ΔT_m value estimated for the bifunctional compound indicates a stronger interaction of the drug with DNA.

In conclusion, BASACV seems to act as a bifunctional intercalator. In this model, the two acridine chromophores are allowed to bind simultaneously to DNA via an intercalative process, since they are positioned in parallel planes at an inter-distance of approx. 1.0 nm (10 Å), as in des-*N*-tetramethylTriostin A. This ideal relative position is imposed by the rigid cyclopeptide backbone, which appears to be a more favourable linker than alkylated bis-acridines previously studied (Wakelin *et al.*, 1978; King *et al.*, 1982). The above preliminary results provide strong evidence that the new acridine analogue of Triostin A, BASACV, possesses a high affinity for DNA. Further studies are required to determine whether the new compound shows a DNA sequence selectivity and/or an enhanced chemotherapeutic effect. In any case, BASACV could be considered as an attractive model with an optimal geometry for study of drug–DNA interaction.

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